INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10480

A. * CLASSIFICATION OF SUBJECT MATTER
IPC(5) :C12Q 1/68; G01N 21/76
US CL :435/6; 436/172
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 436/172

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

(DIALOG (electro chemiluminescen? and (ruthenium or osmium or rhenium))

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, T	US, A, 5,135,717 (RENZONI ET AL.) 04 AUGUST 1992, see entire document.	1-6, 11-14
X	CLINICAL CHEMISTRY, Volume 37, No. 9, issued 1991, G. F. Backburn, et al., "Electrochemical Detection for Development of Immunoassays and DNA Probe Assays for Clinical Diagnosis", pages 1534-1539, see entire document.	1-14
Y	CLINICAL CHEMISTRY, Volume 37, No. 9, issued 1991, J. H. Kenton, et al., "Rapid Electrochemiluminescence Assays of Polymerase Chain Reaction", pages 1626-1632, see entire document.	1-14
A	ANALYTICAL CHEMISTRY, Volume 62, issued 1990, M. Rodriguez et al., "Electrochemical Studies of the Interaction of Metal Chelates with DNA. 4. Voltammetric and Electrogenerated Chemiluminscent Studies of the Interaction of Tris (2,2'-bipyridine) osmium(II) with DNA", pages 2658-2662, see entire document.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
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BIOAFFINITY SENSORS BASED ON ELECTROCHEMILUMINESCENCE

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ABSTRACT

Two types of bioaffinity sensors based on electrochemiluminescence have been developed. The one depends its performance on the electrochemiluminescence of such labels as luminol. Luminol-labeled antibody quenches its electrochemiluminescence by forming immunocomplex with the corresponding antigen (analyte). The electrochemiluminescence is extremely enhanced by hydrogen peroxide. The lower limit of detection is 10^{-12} g ml^{-1} of immunoglobulin G for homogeneous immunoassay. The other is a bioaffinity sensor for primary screening of DNA-interacting chemicals. Ru-complex such as tris-(1,10-phenanthroline) ruthenium (II) is used as an electrocehmiluminescent probe which is intercalated into the major groove of DNA. DNA-interacting chemicals are classified into three groups by the electrochemiluminescence characteristics of the probe.

1. INTRODUCTION

There are two types of biosensors which involve a biocatalytic sensor and a bioaffinity sensor (Fig.1). A biocatalytic sensor is represented by an enzyme sensor which consisting of an enzyme layer on a signal transducing device. Enzyme sensors have been forwarded into third generation of biosensors due to innovative progress specifically in process technology. In contrast a variety of novel principles and technologies have been emerged in development of bioaffinity sensors, which is profoundly stimulated by an enormously increased interest and demand.

Because extremely high sensitivity as well as selectivity is a primary requisite of bioaffinity sensors, they commonly depend their sensitivity on labels that can be detected sensitivity by physical methods. There have been so many labels reported, which contain an enzyme, fluorophor, and electrochemically active substance (Table 1) [1]. We have proposed an electrochemiluminescence substance as label for homogeneous immunoassay [2,3]. In this paper, electrochemiluminescence labels have been applied to not only immunosensing but biosensing of DNA interactive chemicals such as antiviral and antitumor agents.

Luminol is found feasible as label for electrochemilumines-

cence immunosensing because the electrochemiluminescence of antibody-bound luminol is quenched by immunocomplexation with the corresponding antigen. Furthermore, electrochemiluminescence of luminol is markedly enhanced by addition of hydrogen peroxide, which makes the electrochemiluminescence immunosensing highly sensitive.

Ru-complex such as tris-(1,10-phenanthroline) ruthenium (II) generates electrochemiluminescence and is intercalated in the major groove of DNA. No appreciable electrochemiluminescence is detected if the Ru-complex is intercalated in DNA. These characteristics indicate that interaction of DNA with antiviral and antitumor agents could be quantitated by electrochemiluminescence measurement of the Ru-complex as a probe.

There are so many antiviral and antitumor agents that have strong interactions with DNA. Therefore, a simple and quantitative method has been required to be developed for screening DNA-interacting chemicals. In this paper a novel biosensing for primary screening of DNA-interacting chemicals has been developed on the basis of electrochemiluminescence measurement of the DNA-intercalated Ru-complex in the presence of DNA-interacting chemicals.

2. ELECTROCHEMILUMINESCENCE IMMUNOSENSING

Immunoassay with label immunosensors is performed in a heterogeneous manner which requires B/F separation process (Fig.2). Few label immunosensors, however, provide homogeneous immunoassays which are free from B/F (bound and free forms) separation. Since homogeneous immunoassay requires no B/F separation process, intensive research has been made to search for suitable labels which sensitivity change their characteristics in association with immunochemical reactions.

The authors found that electrochemiluminescent substances are promising as label for homogeneous immunoassay (Fig.3), because electrochemiluminescence of luminol and some other substances is effectively quenched by immunocomplexation of antibody labeled with these substances. The analyte is thus quantitatively determined by detecting photons derived from electrochemiluminescence after immunocomplexation. A novel homogeneous immunosensor has been developed on the basis of the electrochemiluminescence quenching of luminol and some other substances by immunocomplexation.

Of these electrochemiluminescence substances luminol is one of the most effective labels for homogeneous immunoassay, because its electrocehmiluminescence generates in a neutral pH and is enhanced by addition of hydrogen peroxide. In the presence of hydrogen peroxide luminol generates electrochemiluminescence by solely electrochemical oxidation. Luminol was selected as a label for electrochemiluminescence homogeneous immunoassay.

Several sensing devices for electrochemiluminescence homogeneous immunoassay have been designed. The one is an optical fiber electrode fabricated with an optically transparent electrode at the terminal of an optical fiber. Another is an thin layer type of electrolytic cell equipped with an optically transparent electrode.

2.1 Eelectrochemiluminescence Measurement

Anti-human immunoglobulin G antibody was labeled by luminol. Diazotized luminol was covalently conjugated with antibody, which was followed by chromatographic purification to isolate a specific molar ratio of conjugate.

Electrochemical luminescence of luminol and antibody-bound luminol was generated on the surface of an optical fiber electrode which was fabricated by forming an optically transparent platinum thin layer at the terminal of an optical fiber (Fig.4). A platinum counter electrode was fabricated around the optical fiber. These electrodes were connected with a potentiostat and a function generator. The optical fiber electrode was led to a photon counter which was connected with a digital storage oscilloscope, computer and printer. The optical fiber electrode and a silver/silver chloride (Ag/AgCl) reference electrode were set in a glass cuvette.

The glass cuvette was filled with a test solution. After deoxygenation by nitrogen gas bubbling, the potential of the optical fiber electrode was controlled with referring to the reference electrode with a potentiostat. Luminescence generated on the electrode surface was quantitated through the photon counting system. Current was also measured if necessary.

A thin layer type of electrolytic cell equipped with an optically transparent electrode was also used for electrochemiluminescence measurement (Fig.5). The optically transparent electrode was made of In_2O_3. The cell contained a platinum counter electrode and a Ag/AgCl reference electrode.

2.2. Electrochemiluminescence of Luminol

Electrochemistry of luminol has been investigated before getting the insight of its electrochemical luminescence. An aqueous solution of luminol was prepared by dissolving luminol in a small portion of DMSO and diluting the resulting solution with a pH 7 of phosphate buffer. Differential pulse voltammetry and cyclic voltammetry have been carried out in the aqueous solution of luminol with the optical fiber electrode of platinum.

Potential was scanned in the range from 0 to 1.0 V vs. Ag/AgCl. Luminol gave a distinctive anodic peak in the potential range as compared with the phosphate buffer. The anodic peak is derived from an electrochemical oxidation of luminol to generate

an active species of luminol. Formation of an active species of luminol was confirmed by the luminol concentration dependency as well as potential scanning rate dependency on the differential pulse voltammetry of luminol.

In order to obtain the electrochemical luminescence of luminol, emitted light was quantitated through the optical fiber electrode while the electrode potential was periodically scanned in the range from -1.5 to + 1.0 V vs. Ag/AgCl. Luminol gave electrochemical luminescence around +0.5 V vs. Ag/AgCl. The electrochemical luminescence required negative potential scanning before the electrode potential was scanned in the positive potential range. It means that the electrochemical oxidation of luminol should be associated by the electrochemical reduction to generate photons.

Cyclic voltammetry in the negative potential range suggested that the electrode potential should be controlled at -1.5 V.vs. Ag/AgCl to generate the electrochemical luminescence of luminol, while the positive electrode potential should be set around 0.5 V vs. Ag/AgCl. Electrochemical luminescence was measured by setting the electrode potential at -1.5 V vs. Ag/AgCl for a certain time, which was followed by shifting the potential at +0.5 V vs. Ag/AgCl. The optimum time duration was thus determined.

Under the optimum conditions, the electrochemical luminescence was measured at different concentrations. Luminol emitted photons intensively enough to be determined even in the concentration range of 10^{-8} mol/l.

In further investigation of the electrochemical luminescence of luminol in an aqueous solution, it was found that electrochemical luminescence of luminol could be extremely assisted by the electrochemical generation of either H_2O_2 or $O_2^{\cdot -}$. The cyclic voltammogram in the negative potential range shows a pair of redox peaks. These peaks seem broad in shape probably due to involvement of several processes. In addition to a reductive process of luminol, some other processes such as reduction of trace amount of O_2 to generate $O_2^{\cdot -}$ and H_2O_2 might be involved.

These results have led us to investigate a possible enhancement of electrochemical luminescence of luminol by addition of hydrogen peroxide, while the reductive potential application is eliminated. As was expected, electrochemical luminescence of luminol was intensively enhanced by addition of hydrogen peroxide and potential application of solely +0.5 V vs. Ag/AgCl. Electrochemical luminescence of luminol increased linearly with an increase of hydrogen peroxide. The electrochemical luminescence might be generated primarily due to the following reaction, although the electrochemically oxidative product of luminol has not been identified.

$$\text{Electrochemically oxidative product of luminol} + H_2O_2 \xrightarrow{+0.5\ V} \text{Product} + \text{Light}$$

Under the condition of potential application of 0.5 V vs. Ag/AgCl and hydrogen peroxide concentration of 1 mmol/l, electrochemical luminescence of luminol was determined at various concentrations of luminol. The lower detection limit of hydrogen peroxide was 10^{-11} mol/l. It is obvious that electrochemical luminescence of luminol was extremely enhanced in presence of hydrogen peroxide.

2.3 Homogeneous Electroluminescence Immunoassay

As an example homogeneous immunoassay was performed for human immunoglobulin G (IgG). A fixed amount of luminol-labeled antibody was added to a solution containing a given concentration of IgG. After immunocomplexation the solution was assayed for its electrochemiluminescence using an electrochemiluminescence immunosensing device equipped with either an optical fiber electrode or optically transparent electrode.

The electrode potential was controlled at 0.8 V vs Ag/AgCl. Hydrogen peroxide was added to the solution to make its concentration at Z mmol/l. A linear standard curve for IgG was determined in the concentration range from 10^{-12} to 10^{-8} g/ml (Fig.6). The lower limit of detection seems 10^{-12} g/ml under the conditions.

3. ELECTROCHEMILUMINESCENT SENSING

A variety of DNA-interacting substances are found effective as antiviral and antitumor agents. An intensive research has been made on synthesizing antiviral and antitumor agents which interact with DNA. It is strongly required to develop a simple method to screen DNA-interacting substances. The authors have proposed that electrochemiluminescence sensing should be feasible in primary screening of DNA-interacting substances.

This -(1,10-phenanthroline) ruthenium (II) (Fig.7) generates electrochemiluminescence upon electrochemical oxidation [4]. The electrochemiluminescence is markedly enhanced in the presence of oxidate. Recently it is shown that one of the phenanthroline ligands intercalates between the base-pairs of double helical DNA [5]. The intercalated Ru-complex stays in the major groove of DNA. It is noted that the intercalated Ru-complex emits no electrochemiluminescence due to sterric hindrance of the complex. The authors have exploited that electrochemiluminescence of the Ru-complex as it can work as a sensing probe for screening the binding mode of DNA-interacting antiviral and antitumor agents (Fig.8).

3.1 ELECTROCHEMILUMINESCENCE SENSING

λDNA was used as a model of double helical DNA. Cisplation,

daunomycin, actinomycin D, chromomycin A3 were used as DNA-interacting agents.

DNA was dissolved in Tris buffer solution, and incubated with each agent, following which the Ru-complex was added. Luminescence of the Ru-complex upon application of applying a stepped potential (0-1.5 V) was measured by a photon-counting system (Fig.9). The resulting luminescence was compared either by changing the amount of DNA or by changing the drug concentration.

3.2 Electrochemiluminescence of the Ru-complex

Electrochemiluminescence of the Ru-complex was measured in the absence and in the presence of oxalate by the potential step excitation. The electrode potential was stepped up from 0 V to 1.5 V vs. Ag/AgCl. The electrode potential was sustained at 0.5 V vs. Ag/AgCl for 10 sec and returned to 0 V vs. Ag/AgCl. The Ru-complex (II) is oxidized to Ru-complex (III) which generates an excited state of Ru-complex (II). Electrochemiluminescence is emitted from the excited state of the Ru-complex (II) while an electron dicipates to either solvent or oxalate radicals.

Electrochemiluminescence of the Ru-complex generated immediately after potential step and decays within 3 sec. Addition of sodium oxalate enhanced electrochemiluminescence of the Ru-complex by the factor of 100 at a concentration of 10 mmol/l (Fig.10).

Electrochemiluminescence of the Ru-complex was sharply depressed by addition of DNA to a solution containing the Ru-complex. Since the Ru-complex is intercalated into the major groove of DNA, electrochemical reaction of the Ru-complex could be inhibited by steric hindrance due to DNA molecular. Consequently, less electrochemiluminescence was generated at higher concentrations of DNA due to intercalation of the Ru-complex into DNA (Fig.11).

3.3 Electrochemiluminescence Screening of DNA-interacting Substances

In the case of cisplatin which is known to bind to DNA tightly, the ECL increased with increase of the amount of agent, because the binding of the Ru-complex to DNA was inhibited due to the tight binding between DNA and cisplatin (Fig.12). This inclination also indicates that both Ru-complex and cisplatin bind to the major groove of DNA.

A similar increase of luminescence was observed, when daunomycin was incubated with λDNA, which indicates that the agent bound to DNA through the major groove, thereby few vacant seats for the Ru-complex was left in the major groove.

On the other hand, no increase in luminescence of the Ru-complex was observed when actinomycin D was incubated. This may

suggest that the drug bound to the minor groove of DNA, therefore, the major groove of DNA has vacant seats for the drug binding, which results in no increase in free Ru-complex.

In summary, DNA-binding modes of the agents are classified to intercalation to double-stranded DNA, binding to the major groove of DNA helix, and binding to the minor groove of the double helix (Fig.13). The present ECL-based sensing was also shown feasible for the estimation of the detailed mode of binding of antiviral or antitumor agent.

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2) Y.Ikariyama, H.Kunoh and M.Aizawa, Biochem.Biophys.Res. Commun., 128, 987 (1985).
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5) M.T.Carter and A.J.Bard, Bioconjugate Chem., 1, 257 (1990).

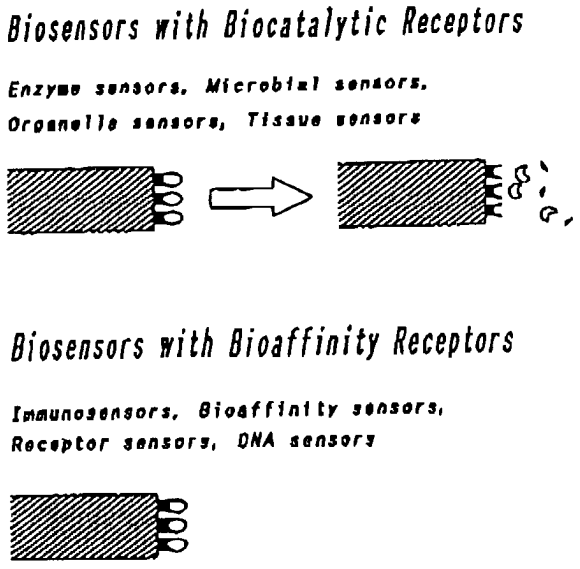


Fig.1 Two types of biosensors

TABLE I

Possible labels for immunosensors

Immunosensors without any label
Electrode potential
Transmembrane potential
Piezoelectric properties
Surface plasmon
Immunosensors with non-isotopic labels
Enzymes:
Catalyse, glucose oxidase
Peroxidase
Alkaline phosphatase
Urease
Catalysts: haemin
Fluorophores
Electrochemically active substances:
Ferrocene,
Pyrene, luminol
Liposomes, erythrocytes

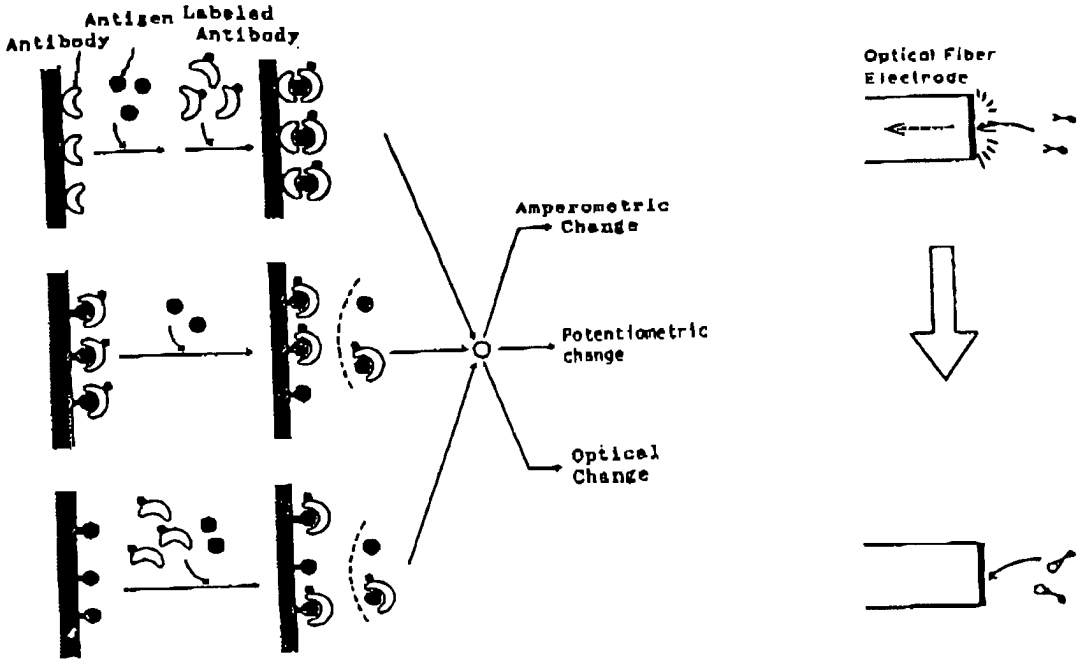
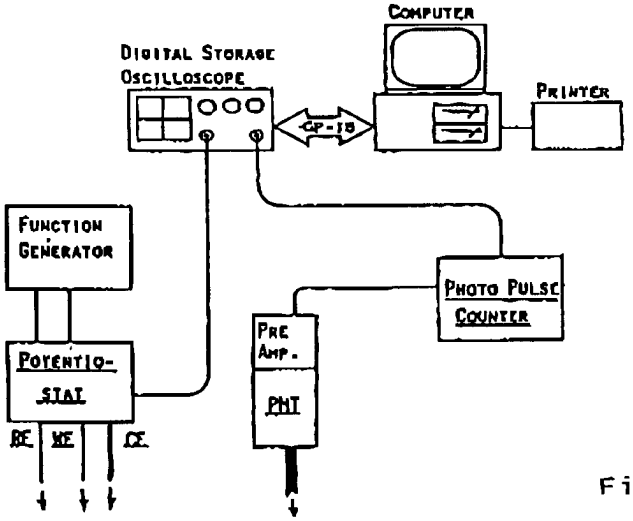


Fig.2 Immunosensors with labels

Fig.3 Homogeneous immunosensing with electrochemiluminescence label



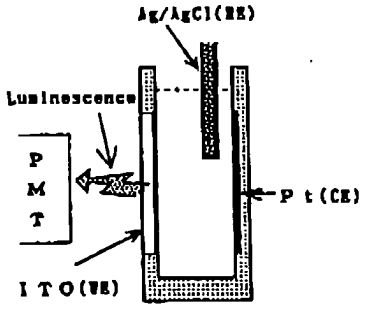


Fig.4 Optical fiber immunosensing system

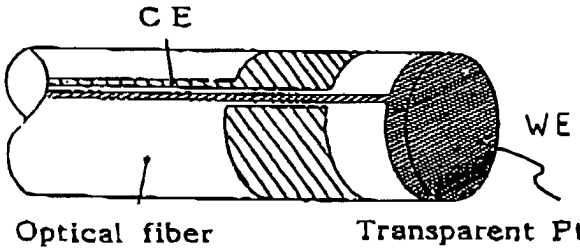


Fig.5 Immunosensing device with optically transparent electrode

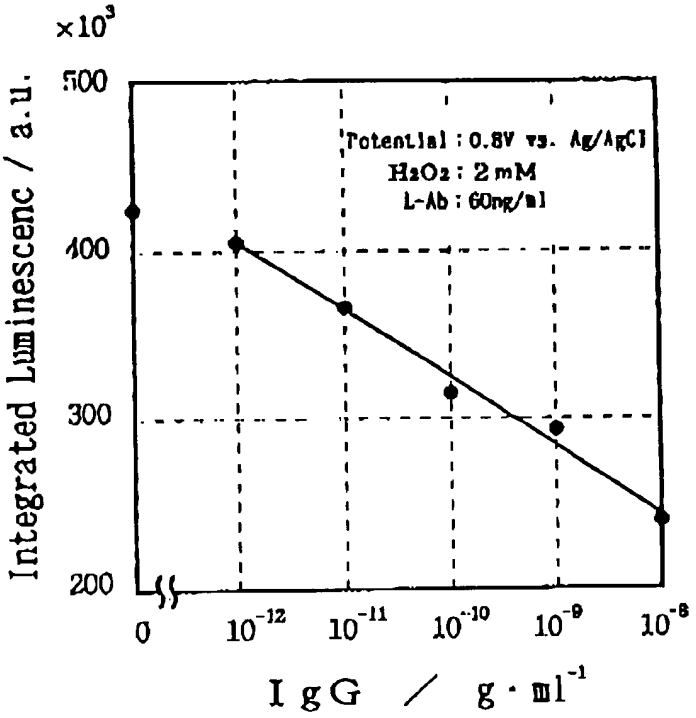


Fig.6 Standard curve for IgG

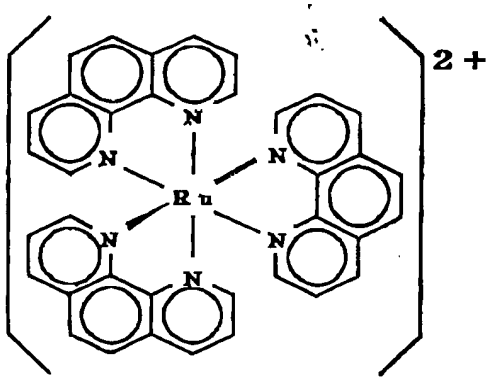


Fig.7 Structure of Ru-complex [II]

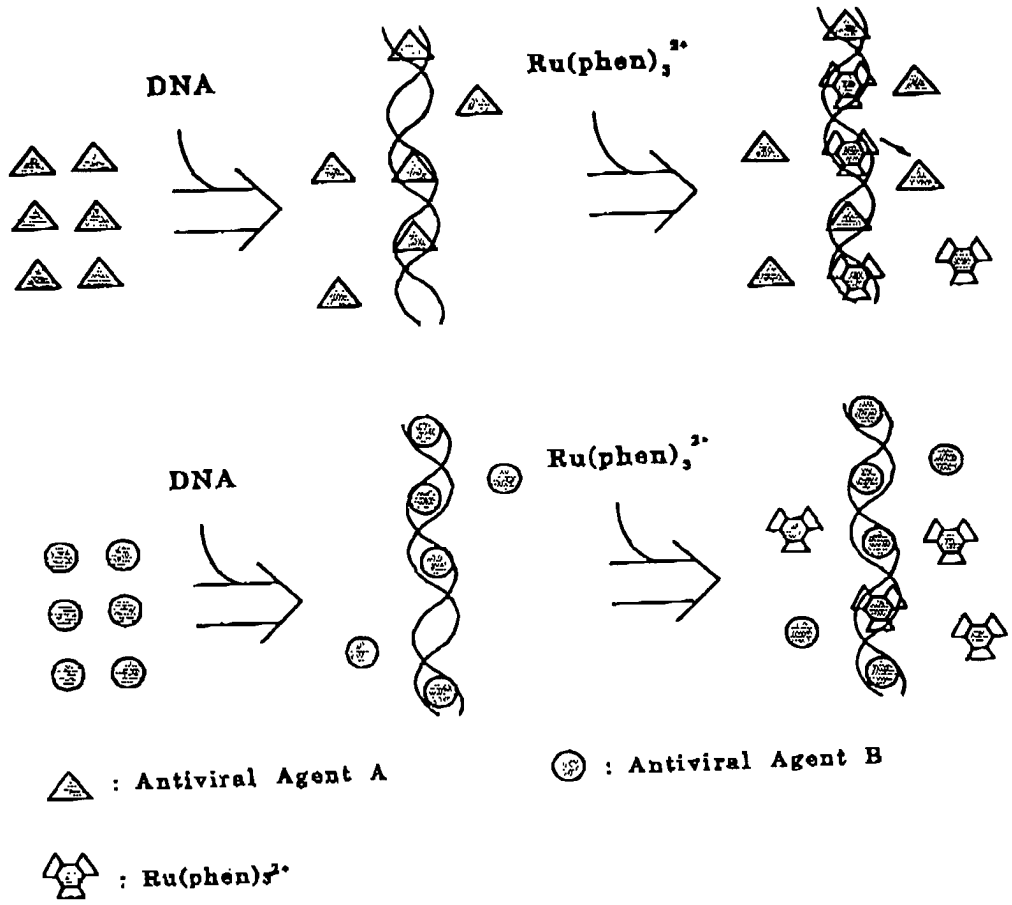


Fig.8 Electrochemiluminescence sensing of DNA-interacting substances with Ru-complex probe

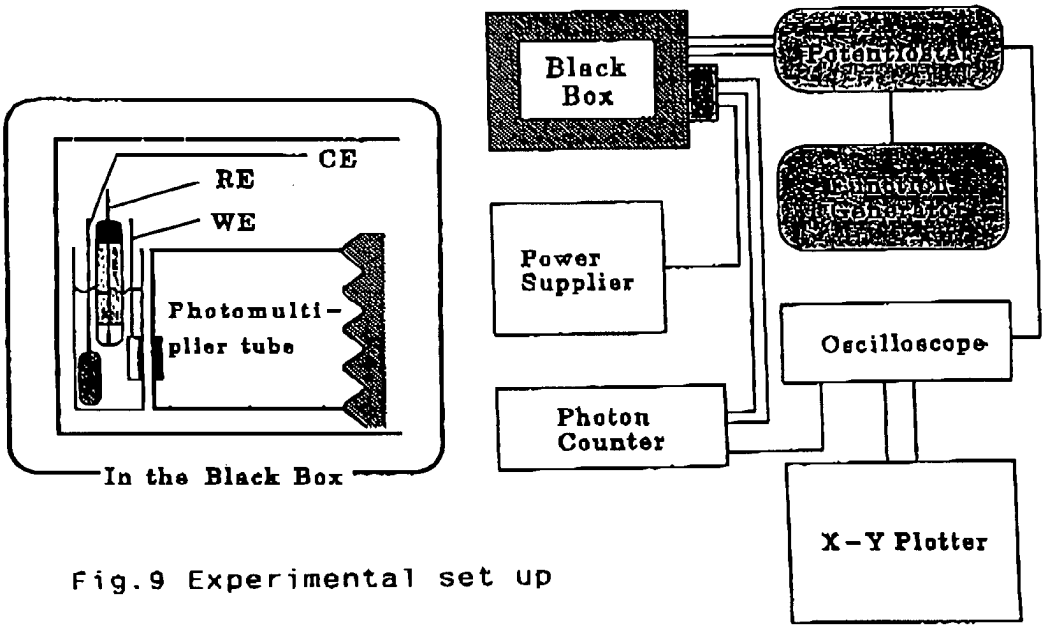


Fig.9 Experimental set up

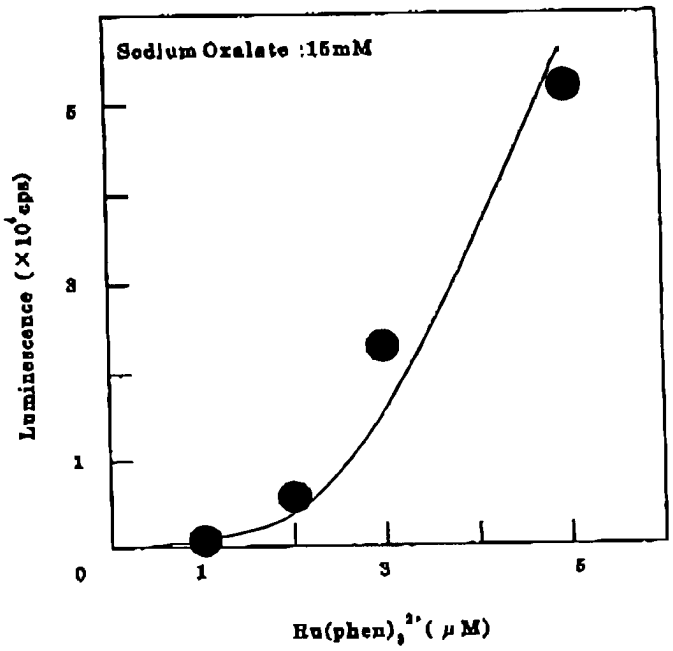


Fig.10 Electrochemiluminescence of Ru-complex in the presence of oxalate

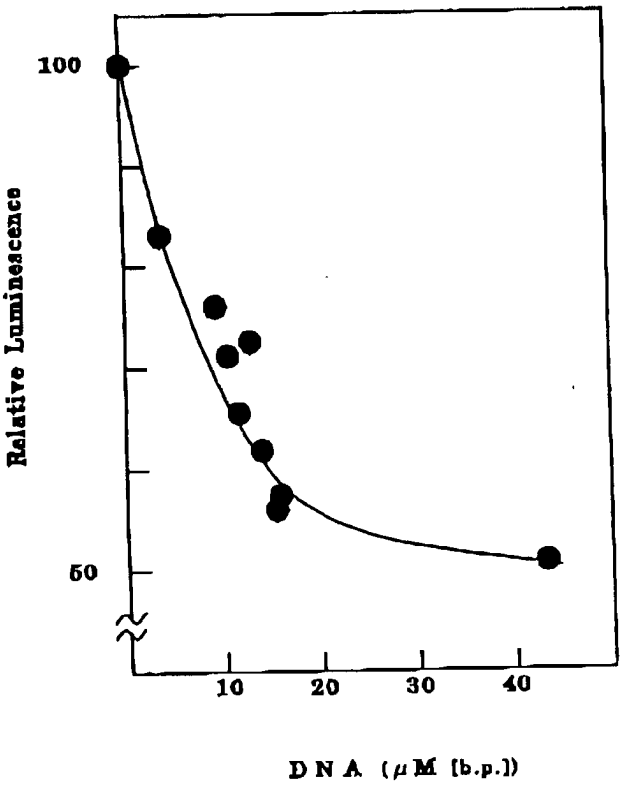


Fig.11 Electrochemiluminescence quenching by DNA

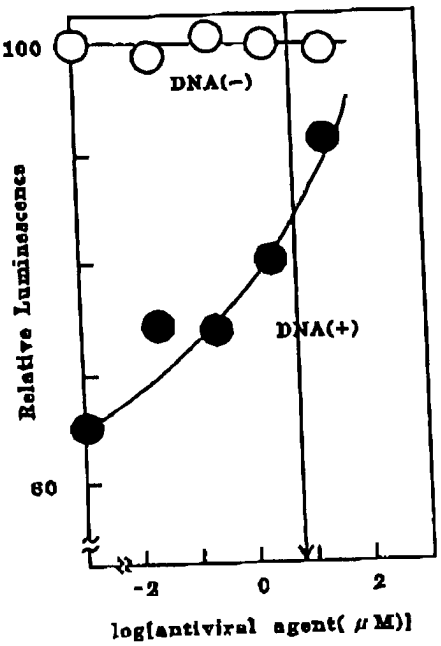


Fig.12 Electrochemiluminescent effect of cis-platin

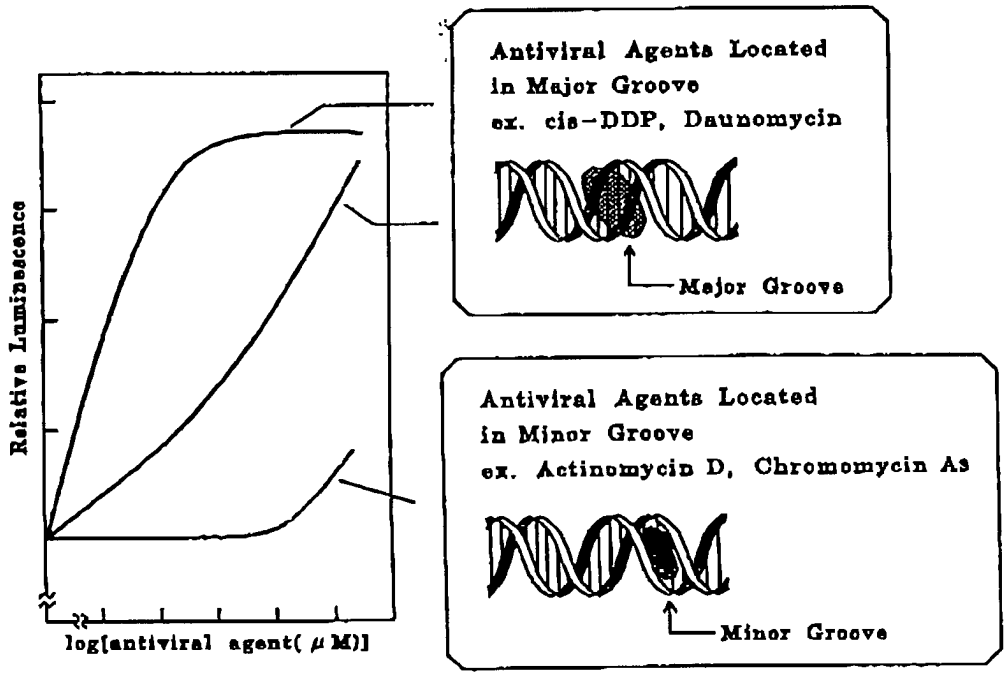


Fig.13 Primary screening of DNA-interacting substances

RESULTS

PRECISION PROFILE

Intra-Run

Sample	Mean (ng/mL)	%CV
1	768	0.4
2	48	6.8
3	3	3.8

Inter-Run

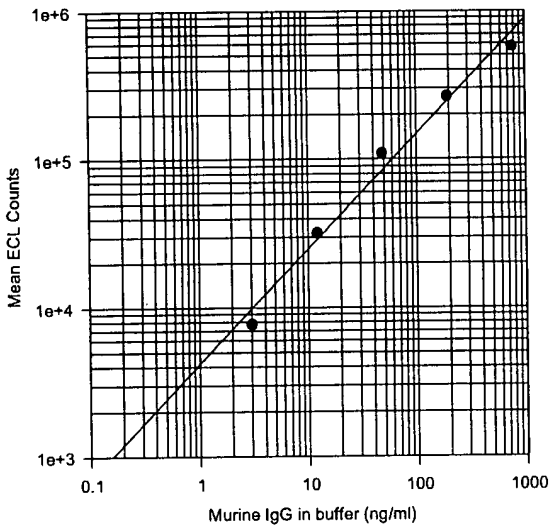
Sample	Mean (ng/ml)	%CV
1	768	0.8
2	192	1.3
3	48	1.9
4	3	0.4

SENSITIVITY

<1 ng/ml

Calculated as zero calibrator plus 2SD

CALIBRATION CURVE



CONCLUSIONS

The assay performed using electrochemiluminescence provides results that are highly precise, rapid, and require no manual wash steps. The rapid turn-around time for the assay coupled with the improved range and precision are key attributes of the system. The increased precision and lower C.V's allow tighter standards to be applied to the QC process which may streamline the FDA approval process.

Electrochemiluminescence allows users flexibility in formatting assays, resulting in the ability to achieve a higher level of performance. The no-wash format and the commercial (IGEN, Inc.) availability of the ruthenylated antibody reduces required labor and reagent waste. Short incubation times and simple protocols allow more experiments to be completed in a given time. Electrochemiluminescence is a powerful new research tool with demonstrated superior performance in this murine IgG assay.

PERFORMANCE SUMMARY	
PRECISION	<10% C.V.
CALIBRATION RANGE	3-768 ng/ml
STEPS IN ASSAY	1
WASHES	0
INCUBATION TIME	1 hour

IGEN International, Inc.

1-800-336-4436
http://www.igen.com

DETECTION AND QUANTITATION OF JC VIRUS

INTRODUCTION

JC Virus (JCV), which is a human polyomavirus, is the etiologic agent of the human demyelinating disease, progressive multifocal leukoencephalopathy (PML). Until recently, PML was a rare disease; however, with the increase in the incidence of AIDS, there has been an increase in the number of cases of PML. With the introduction of recent amplification technologies, the presence of JCV DNA can be detected in the peripheral blood lymphocytes (PBL) and urine of a large portion of the non-symptomatic adult population. In order to confirm the diagnosis of PML, the presence of JCV must be noted in specific tissue(s) of the patient. In addition, it has become of clinical interest to monitor the presence of JCV in these tissues of patients at risk for PML.

Electrochemiluminescence, a technology developed by IGEN, Inc. and employed in the ORIGEN Analyzer, offers superior assay performance in a flexible easy to use format. Electrochemiluminescence technology utilizes an extremely stable ruthenium metal chelate (TAG) which, in the presence of tripropylamine (TPA), participates in a luminescent reaction that is triggered by the application of an electric potential. Flexible formatting allows sandwich, direct, and competitive assay designs for use in immunoassays, quantitation of nucleic acids, receptor-ligand binding, and measurement of particulate matter such as viruses and bacteria. Paramagnetic beads, microns in diameter, act as the solid phase and facilitate rapid assay kinetics. The bead/complex is channeled through a uniquely designed flow cell and captured at the surface of an electrode by magnetic application. Voltage is applied and resulting luminescence is measured. This process employed by the ORIGEN Analyzer eliminates wash steps, minimizes incubation time, while producing assay results equivalent or superior to currently available methods.

Current procedures to detect and identify the presence of JCV requires the amplification of the patient's sample with specific primers, analysis of the amplified products by gel electrophoresis, and Southern blot hybridization to confirm the presence JCV DNA sequences. This procedure in most cases would take a minimum of three days to confirm the presence of JCV. Development of an electrochemiluminescence (ECL) assay detecting the presence of JCV sequences established several advantages over the present conventional methodology. The ECL assay is a nonisotopic procedure. The assay confirming the presence of JCV DNA sequences is significantly shorter. Electrochemiluminescence is as sensitive an assay as the isotopic procedure. This note describes the essential findings of the development of an ECL assay to detect the presence of JCV DNA sequences in patient samples.

MATERIAL AND METHODS

PREPARATION OF DNA FROM SAMPLES

PBL's were purified by conventional techniques utilizing a Ficoll-Hypaque gradient, treated with 1% SDS and 100 ug of Proteinase K, extracted with phenol/chloroform, and ethanol precipitated. The DNA was suspended in 200 ul of 1X TE. Cerebral spinal fluid (CSF) was heated at 100°C for 15 minutes before use.

AMPLIFICATION REACTION

The 5' JCV primer, which is biotinylated, is from the JCV early region spanning nucleotides 4561 to 4580. The 3' JCV primer is derived from nucleotides 4719 to 4700. The amplification reaction mixture (100 µl) contained the following components: 10 mM Tris HCl, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$; 0.01% gelatin; 0.2 mM of dNTPs; 10 pmoles of each primer; and various amounts of template. DNA from PBL's and tissues were used at 0.5 to 1.0 ug. Five to 10 µl of CSF were used per reaction. Reaction mixtures were overlayed with 50 ul of mineral oil before incubation. The samples were amplified using the following program: 95°C for 1 minute; 55°C for 1 minute; 72°C for 2 minutes; 35 cycles, and extended at 72°C for 7 minutes.

GEL ELECTROPHORESIS AND SOUTHERN BLOT HYBRIDIZATION

Amplified products (10 µl) were examined on a 6% polyacrylamide gel with 1X TAE buffer. For Southern blot hybridization studies, samples (10-40 µl) were analyzed on 1.0-1.4% agarose gels using 1X TBE and transferred by standard procedures. Approximately 1 X 10^6 cpm/ml of ^{32}P-labeled nick translated JCV DNA (pMad1) was used as the probe and incubated at 42°C for 15-18 hrs. Autoradiographs were developed after 1-13 days of exposure.

ORIGEN DETECTION SYSTEM

Amplified samples (5 µl) in triplicate were mixed with 20 µg of M280 streptavidin coated Dynabeads® (Dynal, A.S.) in a final volume of 50 µl of PBS 0.1% Tween 20 and incubated for 15 min. Samples were denatured in 200 µl of 0.1N NaOH, washed in 250 µl of PBS Tween, and probed with the ORIGEN TAG'd (JCV nucleotides 4630-4657) 10 ng in 50 µl of Assay Buffer at 42°C for 30 min. Samples were read in the ORIGEN Analyzer after the addition of 250µl of ORIGEN Assay Buffer.

The ORIGEN TAG oligonucleotide was prepared using 5' amino oligonucleotide and ORIGEN TAG-NHS Ester following the recommended procedure in the application note.

RESULTS

The diagram below (Figure 1) shows one format used in this study to detect the presence of specific JCV DNA sequences in amplified samples.

Figure 2A shows an example of samples after amplification of standardized amounts of JCV DNA under the conditions stated in Materials and Methods. Figure 2B shows the ECL units obtained from these same samples using the assay format shown in Figure 1. Figure 2C shows the plot of the ECL units. Note the dynamic range of close to 3 orders of magnitude.

Table I shows an example of some results from a study analyzing different types of patients specimens for the presence of JC virus. Note the high correlation between the ECL results and the Southern hybridization results.

CONCLUSIONS

This study demonstrates that the detection of JC virus by electrochemiluminescence with the ORIGEN® Analyzer was as sensitive as the standard isotopic procedure. Additionally, the ORIGEN Detection System provides objective, quantitative results read by a calibrated instrument increasing the precision of results over time.

In addition, electrochemiluminescence offers a number of advantages over the standard detection procedure:

1) Non-radioactive, long lasting detection probes,
2) Savings of one to two days in obtaining results, and
3) Efficiency; requiring less material and manipulations to execute.

In summary, quantitation of nucleic acid hybridizations is highly simplified using the ORIGEN Detection System. In this case, coupling of the ORIGEN TAG label to oligonucleotides allows them to be used as specific hybridization and detection probes. The assay described above demonstrates the flexibility and speed with which the ORIGEN System can be applied for accurate quantitation of DNA hybridization assays.

Warnings and Limitations: This product is optimized for use in the Polymerase Chain Reaction ("PCR") covered by patents owned by Hoffmann-La Roche Inc. and F. Hoffmann-La Roche Ltd. ("Roche"). No license under these patents to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of this product. A license to use the PCR process for certain research and development activities accompanies the purchase of certain reagents from licensed suppliers or may be obtained by contacting the Perkin-Elmer Corporation, 850 Lincoln Center Drive, Foster City, California 94404 or Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

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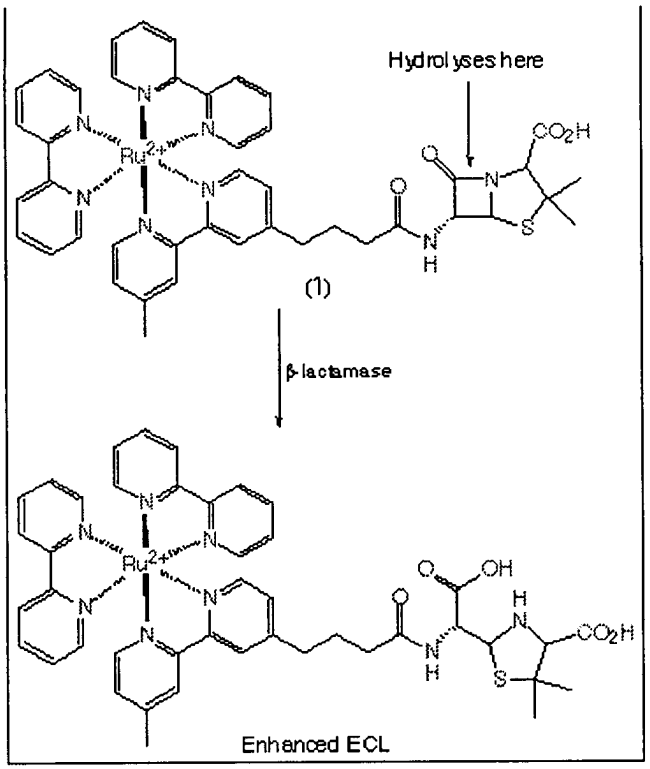
CHEMISTRY & INDUSTRY Highlights

Electrochemiluminescence to detect beta-lactamases

Electrochemiluminescence (ECL) is a phenomenon that occurs when stable compounds are electrochemically modified to form relatively unstable intermediates that can combine to produce light. An archetypal example of this effect is the simultaneous oxidation of an amine and $Ru(bipy)_3^{2+}$, which leads to the ejection of a 620 nm photon from the ruthenium centre. Combination of the reactive species formed after oxidation is pivotal in all ECL reactions; light emission cannot occur if decomposition of the reactive intermediates happens before they encounter each other. In a recent issue of J. Am. Chem. Soc., researchers from IGEN report electrochemiluminescence to detect beta-lactamases (Liang, Dong and Martin, 1996, 118, 9198). This is important because beta-lactamases are excreted by cells that have a resistance to beta-lactam antibiotics, and resistance to these antibiotics is a ubiquitous problem. The IGEN researchers noted that hydrolysed penicillins are much more effective partners in ECL reactions than their unhydrolysed forms. They then optimised the probability of ECL detection by covalently linking the non-hydrolysed penicillins to $Ru(bipy)_3^{2+}$. Compound (1) is an example of the systems they prepared. Hydrolysis of the beta-lactam functionality of (1) is mediated by beta-lactamases, hence the presence of these enzymes can be inferred via ECL detection measurements.



18 November 1996
Kevin Burgess, Texas A&M University

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Electrochemiluminescence: 1996–1998

Compiled by L. J. Kricka and P. E. Stanley

The journal continues to provide comprehensive literature surveys which will be published in most issues. These are a continuation of the literature surveys begun in 1986 in the *Journal of Bioluminescence and Chemiluminescence*, and which have up to 1998, encompassed more than 6000 references cited by year or specialized topic.

With this newly named Journal these searches are expanded to reflect the Journal's wider scope. In future we will cover all fundamental and applied aspects of biological and chemical luminescence and include not only bioluminescence and chemiluminescence but also fluorescence, time-resolved fluorescence, electrochemiluminescence, phosphorescence, sonoluminescence, lyoluminescence and triboluminescence, etc.

The compilers would be pleased to receive any comments from the readership. Contact by e-mail: L. J. Kricka (larry_kricka@path1a.med.upenn.edu), and P. E. Stanley (stanley@LUMIWEB.COM).

1996

Electrochemiluminescence – a new technology system		FASEB J 1996;10:T15
Determination of binding constants of diabodies directed against prostate-specific antigen using electrochemiluminescence-based immunoassays	Abraham R, Buxbaum S, Link J, Smith R, Venti C, Darsley M	J Molec Recog 1996;9:456–61
The application of the electrochemiluminescence method for studying the mechanism of electrode-reactions complicated by subsequent chemical stages—mechanisms of the oxidation reaction and anodic electrochemiluminescence in systems containing sodium tetraphenylborate	Belash EM, Rozhitskii NN	Russ J Electrochem 1996;32:1263–72
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A fully automated electrochemiluminescent CA125II™ assay	Bergey B, Welham D, Hofler J, Clarke J	Clin Chem 1996;42:S256 Abstract 670
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Electrochemiluminescence from tunicate, tunichrome–metal complexes and other biological samples	Bruno JG, Collard SB, Kuch DJ, Cornette JC	J Biolumin Chemilumin 1996;11: 193–206
Immunomagnetic-electrochemiluminescent detection of Bacillus anthracis spores in soil matrices	Bruno JG, Yu H	Appl Environ Microbiol 1996;62: 3474–6
Gas-phase chemical sensing using electrochemiluminescence	Collins GE	Sensor Actuator B-Chem 1996;35: 202–6

CCC 1522–7235/99/020113–06 $17.50

Enzyme-triggered formation of electrochemiluminescent ruthenium complexes	Dong LW, Martin MT	Anal Biochem 1996;236:344–7
Electrochemiluminescence of hydroxyl compounds by cyclic square-wave electrolysis and its application to an alcohol sensor	Egashira N, Nabeyama Y, Kurauchi Y, Ohga K	Anal Sci 1996;12:793–5
System evaluation of electrochemiluminescence immunoassay analyzer Elecsys®-1010	Finke W, Furst O, Jansen P, Meyer HD	Clin Chem 1996;42:193
Electrochemiluminescence – leading-edge technology for automated immunoassay analyte detection	Hoyle NR, Erkert B, Kraiss S	Clin Chem 1996;42:1576–8
A microfabricated, electrochemiluminescence cell for the detection of amplified DNA	Hsueh YT, Smith RL, Northrup MA	Sensor Actuator B-Chem 1996;33: 110–4
Electrochemiluminescence-based quantitation of classical clinical-chemistry analytes	Jameison F, Sanchez RI, Dong LW, Leland JK, Yost D, Martin MT	Anal Chem 1996;68:1298–302
Development of an electrochemiluminescent immunoassay for digoxin	Kheradmand M, Huber E, Pappert G, Chang CC	Clin Chem 1996;42:381
Electrochemical detection in bioanalysis	Kissinger PT	J Pharmaceut Biomed Anal 1996;14:871–80
A comparison of electrochemiluminescence and flow-cytometry for the detection of natural latex-specific human-immunoglobulin-E	Kobrynski L, Tanimune L, Pawlowski NA, Douglas SD, Campbell DE	Clin Diag Lab Immunol 1996;3:42–6
An electrochemiluminescence immunoassay for the quantitative determination of ferritin using the random-access analyzer Elecsys®	Kyriatsoulis A, Feldmann S, Kraus W, Papper G	Clin Chem 1996;42:75
An electrochemiluminescence immunoassay for the quantitative determination of cyfra-21-1 using the random-access analyzer Elecsys®	Kyriatsoulis A, Waechter N, Kraus W, Papper G	Clin Chem 1996;42:656
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Electrochemistry of aromatic diselenides and ditellurides in aprotic media – preceding formation of mercury-containing compounds	Ludvik J, Nygard B	Electrochim Acta 1996;41:1661–5
Field-study of thyroid-function tests on the Elecsys® electrochemiluminescent immunoassay system	Luppa P, Huber U, Neumeier D, Kraiss S	Clin Chem 1996;42:332
Quantification of cytokine messenger-RNA in transfected human t-cells by rt-PCR and an automated electrochemiluminescence-based post-PCR detection system	Motmans K, Raus J, Vandevyver C	J Immunol Methods 1996;190:107–16

An electrochemiluminescent immunoassay for the determination of free prostate-specific antigen (FPSA) using the Elecsys® analyzer	Moyer V, Laffineuse L, Kenten E, Mossner E, Saul R	Clin Chem 1996;42:692
Micellar halide and energy-transfer effects in electrochemiluminescence	Rypka M, Lasovsky J	J Electroanal Chem 1996;416:41–5
Evaluation of electrochemiluminescence- and bioluminescence-based assays for quantitating specific DNA	Siddiqi AM, Jennings VM, Kidd MR, Actor JK, Hunter RL	J Clin Lab Anal 1996;10:423–31
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A comparative study of fluorescence and enhanced electrochemiluminescence in immuno- and nucleic acid-based assays	Yu H	Prog Biophys Mol Biol 1996;65:PD204
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Immunomagnetic-electrochemiluminescent detection of Escherichia coli O157 and Salmonella typhimurium in foods and environmental water samples	Yu H, Bruno JG	Appl Environ Microbiol 1996;62: 587–92

1997

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Generation of free radicals and electrochemiluminescence at pulse-polarized oxide-covered silicon electrodes in aqueous solutions	Alakleme T, Kulmala S, Latva M	Acta Chem Scand 1997;51:541–6
Sub-microliter electrochemiluminescence detector – a model for small volume analysis systems	Arora A, Demello AJ, Manz A	Anal Commun 1997;34:393–5
Further characterization of tunicate and tunichrome electrochemiluminescence	Bruno JG, Collard SB, Andrews ARJ	J Biolumin Chemilumin 1997;12: 155–64
An electrochemiluminescence assay based on the interaction of diaminotoluene isomers with gold(I) and copper(II) ions	Bruno JG, Cornette JC	Microchem J 1997;56:305–14
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Study of the electrochemiluminescence based on the reaction of hydroxyl compounds with ruthenium complex	Chen X, Jia L, Wang XR, Hu GL, Sato M	Anal Sci 1997;13:71–5
Electrochemiluminescence and efficient light emitting diodes based on multifunctional dyes: donor/acceptor substituted stilbenoids and difluoroboradiaza-s-indacenes	Daub J	Amer Chem Soc. Abstracts of Papers 1997;213:170
First results from external evaluation of electrochemiluminescence analyzer Elecsys® 1010	Fuerst O, Beyrau R, Gurr E, Hoff T, Mclawhon RW, Meyer HD et al.	Clin Chem 1997;43:121–8
Development of an equilibrium immunoassay using electrochemiluminescent detection for a novel recombinant protein product and its application to pre-clinical product development	Grimshaw C, Gleason C, Chojnicki E, Young J	J Pharm Biomed Anal 1997;16:605–12
Energetic electrochemiluminescence of (9-fluorenyl)methanol induced by injection of hot electrons into aqueous electrolyte solution	Kulmala S, Alakleme T, Heikkila L, Vare L	J Chem Soc, Faraday Trans 1997;93:3107–13
Ultrasonic modification of light emission from electrochemiluminescence processes	Malins C, Vandeloise R, Walton D, Vanderdonckt E	J Phys Chem A 1997;101:5063–8
A highly sensitive electrochemiluminescence immunoassay for interferon alfa-2b in human serum	Obenauerkutner LJ, Jacobs SJ, Kolz K, Tobias LM, Bordens RW	J Immunol Methods 1997;206:25–33
Cytomegalovirus glycoprotein B-specific antibody analysis using electrochemiluminescence detection-based techniques	Ohlin M, Silvestri M, Sundqvist VA, Borrebaeck CA	Clin Diagn Lab Immunol 1997;4: 107–11
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Electrochemiluminescence flow injection immunoassay for atrazine	Wilson R, Barker MH, Schiffrin DJ, Abuknesha R	Biosens Bioelectron 1997;12:277–86
Optimization of electrochemiluminescence immunoassay for sensitive bacteria detection	Yu H	ACS Symp Ser 1997;657:162–9
Electrochemiluminescence from calixarene-coated porous Si liquid junction cells	Zhang LB, Coffer JL	J Phys Chem B 1997;101:6874–8
Electrochemiluminescence from calixarene-coated porous silicon liquid junction cells	Zhang LB, Coffer JL	Amer Chem Soc. Abstracts of Papers 1997;213:353

Modulation of amyloid precursor protein processing by compounds with various mechanisms of action: detection by liquid phase electrochemiluminescent system	Khorkova OE, Patel K, Heroux J, Sahasrabudhe S	J Neurosci Meth 1998;82:159–66
Troponin t and creatinine kinase-mb by electrochemiluminescence immunoassay compared with troponin I	Kim JH, Shin HB, Park KI, Lee HM, Kwon OH, Kim DS	Clin Chem 1998;44:539
Electrochemistry of ITO and gold electrodes covalently modified with dithizone and the effect of Cu(II) and Pb(II) ions	Mirkhalaf F, Whittaker D, Schiffrin DJ	J Electroanal Chem 1998;452:203–13
An electrochemiluminescent assay for the determination of IgE using the Elecsys® assay system	Moyer V, Burdette M, Clarke D, Gallusser A, Hallermayer K, Kenten E et al.	Clin Chem 1998;44:2
Detection of tyrosinase mRNA in melanoma by reverse transcription-PCR and electrochemiluminescence	O'Connell CD, Juhasz A, Kuo C, Reeder DJ, Hoon DS	Clin Chem 1998;44:1161–9
Electrochemical formation of luminescent convective patterns in thin-layer cells	Orlik M, Rosenmund J, Doblhofer K, Ertl G	J Phys Chem B 1998;102:1397–403
Clay-enhanced electrochemiluminescence and its application in the detection of glucose	Ouyang CS, Wang CM	J Electrochem Soc 1998;145:2654–9
A possible scheme of electrochemiluminescence generation on platinum cathodes in aqueous solutions of peroxydisulfates	Reshetnyak OV, Kovalchuk EP	Electrochim Acta 1998;43:465–9
Electrochemically oxidized ferrocenes as catalysts for the chemiluminescence oxidation of luminol	Wilson R, Schiffrin DJ	J Electroanal Chem 1998;448:125–30
Electrochemiluminescence oxalic acid sensor having a platinum electrode coated with chitosan modified with a ruthenium (II) complex	Zhao CZ, Egashira N, Kurauchi Y, Ohga K	Electrochim Acta 1998;43:2167–73

INTERLEUKIN 8 IN HUMAN SERUM
A Highly Sensitive and Wide Dynamic Range Assay

ORIGEN TECHNOLOGY

Electrochemiluminescence, a technology developed by IGEN® International, Inc. and employed in the ORIGEN® 1.5 Analyzer, offers superior assay performance in a flexible easy to use format. Electrochemiluminescence technology utilizes an extremely stable ruthenium metal chelate (TAG) which, in the presence of tripropylamine (TPA), participates in an electrochemiluminescent reaction that is triggered by voltage application. Flexible formatting allows sandwich, direct, and competitive assay designs for use in immunoassays, quantitation of nucleic acids, receptor-ligand binding, and measurement of viruses and bacteria. Paramagnetic beads, microns in diameter, act as the solid phase and facilitate rapid assay kinetics. The bead/complex is channeled through a uniquely designed flow cell and captured at the surface of an electrode by magnetic application. Voltage is applied and resulting electrochem-iluminescence is measured. This process employed by the ORIGEN Analyzer eliminates wash steps and minimizes incubation time, while producing assay results equivalent or superior to currently available methods.

DESCRIPTION

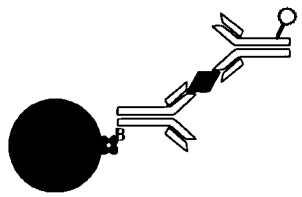
Interleukin 8 (IL-8) is released by a variety of cells, some of which include fibroblasts, epithelial cells, smooth muscle cells, and endothelial cells, as a response to the primary cytokines IL-1 and TNF-α. Some viruses and some tumor cell lines can act as inducers of IL-8. Originally, IL-8 was discovered and purified as a neutrophil chemotactic and activating factor. IL-8 has a wide range of other pro-inflammatory effects. There have been an increasing number of reports on the in vivo release of IL-8 during inflammation and infection, such as lesions of psoriasis patients.

This application review describes the use of electrochemiluminescence and the ORIGEN Analyzer to improve the sensitivity of assays used for detection of IL-8 in human serum. The assay was formatted using an easy two-step, no wash protocol which provides results that are sensitive (1.8 pg/ml), reproducible (inter-assay <8% C.V.), and precise (intra-assay <15% CV), across a wide range of concentrations (9.8-10,000 pg/mL).

MATERIALS AND METHODS

Human IL-8 was purchased from R & D Systems and solubilized in sterile PBS, pH 7.4, containing 0.1% (w/v) bovine serum albumin. The standards were prepared in normal human serum (Rockland, Inc.). The ruthenylated goat anti-human IL-8 antibody was diluted to 4 µg/ml and the biotinylated mouse anti-human IL-8 monoclonal antibody was diluted to 1 µg/ml in an optimized diluent of PBS, pH 7.8, containing 1.5% (w/v) Tween-20 and 0.5% (v/v) normal goat serum (Valley Biomedical). Streptavidin coated M280 Dynabeads® (IGEN, Inc.) were diluted in the same diluent to a concentration of 1.4 mg/ml. Twenty-five µl of each IL-8 standard, and both antibodies, were shaken for two hours at room temperature. Twenty-five µl of streptavidin coated beads were then added and shaken for an additional 30 minutes at room temperature. The reaction was quenched by the addition of 500 µl of ORIGEN Assay Buffer (IGEN, Inc.) and the bound immune complexes were quantitated on the ORIGEN 1.5 Analyzer with the default settings except for an average analysis and a ten second bead wash time.

FORMAT



Streptavidin-coated Beads

PROTOCOL

- 25 µL Sample
- 25 µL Ruthenylated Antibody
- 25 µL Biotinylated Monoclonal Antibody

⇓ Incubate two hours @ room temp

- 25 µL Streptavidin M280 beads

⇓ Incubate 30 minutes @ room temp

- Dilute with 500 µL of Assay Buffer

≠ Read assay

RESULTS

PRECISION PROFILE

Intra-Run

Sample	Mean (pg/mL)	%CV
1	10,000	0.6%
2	625	1.0%
3	9.8	0.8%

Inter-Run

Dose (pg/ml)	Calc. Dose (pg/ml)	%CV
10,000	10022.3	0.2%
625	627.7	0.6%
9.8	9.85	1.9%

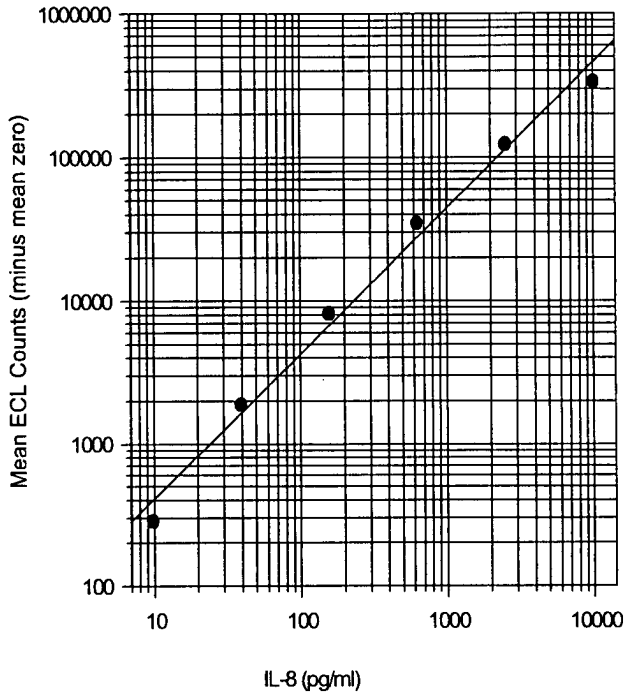
Determined by evaluation of samples in duplicate for 3 experiments

SENSITIVITY

1.8 pg/ml

Calculated as zero calibrator plus 2 S.D.

CALIBRATION CURVE



CONCLUSIONS

The ORIGEN IL-8 assay offers a very stable, reproducible, easy to use, and sensitive assay. The short incubation times and simple assay protocol simplifies operation and execution of the assay increasing the efficiency of the laboratory and allowing more research to be accomplished in less time. Electrochemiluminescence allows users flexibility in formatting assays; providing the capability to achieve a higher level of performance. The no wash format reduces required labor and waste. Fast, accurate, and highly reproducible assays will certainly benefit those investigators working towards new discoveries. Electrochemiluminescence is a powerful new research tool with demonstrated superior performance in an IL-8 assay.

PERFORMANCE SUMMARY

PRECISION	<15% C.V.
CALIBRATION RANGE	9.8-10,000 pg/mL
STEPS IN ASSAY	2
WASHES	0
INCUBATION TIME	2.5 hours

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Substrate selectivity of an electrochemiluminescence Pt electrode coated with a $Ru(bpy)_3^{2+}$-modified chitosan silica gel membrane	Zhao CZ, Egashira N, Kurauchi Y, Ohga K	Anal Sci 1997;13:333–6

1998

Preliminary studies of the enhanced electrochemiluminescence of 2,3-diaminonaphthalene in the presence of specific metal ions	Bruno JG	J Biolumin Chemilumin 1998;13: 139–45
Preliminary electrochemiluminescence studies of metal ion-bacterial diazoluminomelanin (DALM) interactions	Bruno JG, Parker JE, Holwitt E, Alls JL, Kiel JL	J Biolumin Chemilumin 1998;13: 117–23
A study on electrochemiluminescence of $[Ru(bpy)(2)(1\text{-}Trp)]ClO_4$ complex in water solution	Chen GN, Lin RE, Xie ZH, Duan JP, Zhang L	Acta Chimica Sinica 1998;56:433–8
Study of the electrochemiluminescence based on tris(2,2′-bipyridine) ruthenium(II) and alcohols in a flow injection system	Chen X, Sato M, Lin YJ	Microchem J 1998;58:13–20
Results of the multicentre evaluation of an electrochemiluminescence immunoassay for HCG on Elecsys® 2010	Ehrhardt V, Assmann G, Batz O, Bieglmayer C, Muller C, Neumeier D *et al.*	Wien Klin Wochenschr 1998;110:61–7
Highly efficient solid-state electrochemically generated chemiluminescence from ester-substituted trisbipyridineruthenium(II)-based polymers	Elliott CM, Pichot F, Bloom CJ, Rider LS	J Am Chem Soc 1998;120:6781–4
Elecsys® immunoassay systems using electrochemiluminescence detection	Erler K	Wien Klin Wochenschr 1998;110:5–10
Scanning electrochemical microscopy. 37. Light emission by electrogenerated chemiluminescence at secm tips and their application to scanning optical microscopy	Fan FRF, Cliffel D, Bard AJ	Anal Chem 1998;70:2941–8
Evidence for laser action driven by electrochemiluminscence	Horiuchi T, Niwa O, Hatakenaka N	Nature 1998;394:659–61
The assessment of bone metabolic status with serum c-telopeptide, PTH & osteocalcin: three electrochemiluminescent immunoassays for the fully automated analysis of bone turnover	Hoyle NR, Kyriatsoulis A	Clin Chem 1998;44:638
An electrochemiluminescent assay for the determination of myoglobin using the Elecsys®, assay system	Kenten E, Burdette M, Freeman H, Gallusser A, Hallermayer K, Hunter A *et al.*	Clin Chem 1998;44:548

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MURINE IgG IN BUFFER
A rapid, one-step assay

ORIGEN TECHNOLOGY

Electrochemiluminescence, a technology developed by IGEN International, Inc. and employed in the ORIGEN Analyzer, offers superior assay performance in a flexible easy to use format. Electrochemiluminescence technology utilizes an extremely stable ruthenium metal chelate (TAG) which, in the presence of tripropylamine (TPA), participates in a luminescent reaction that is triggered by the application of an electric potential. Flexible formatting allows sandwich, direct, and competitive assay designs for use in immunoassays, quantitation of nucleic acids, receptor-ligand binding, and measurement of viruses and bacteria. Paramagnetic beads, microns in diameter, act as the solid phase and facilitate rapid assay kinetics. The bead/complex is channeled through a uniquely designed flow cell and captured at the surface of an electrode by magnetic application. Voltage is applied and resulting luminescence is measured. This process employed by the ORIGEN Analyzer eliminates wash steps, minimizes incubation time, while producing assay results equivalent or superior to currently available methods.

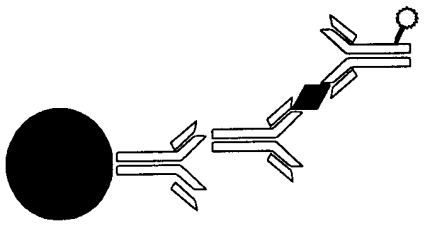
DESCRIPTION

Quantitation of either potentially contaminating immunoglobulins (IgG, IgM, etc.) in a bioproduct process or of an immunoglobulin product is becoming increasingly important to the pharmaceutical/biotechnology industry. This application review describes the use of electrochemiluminescence and the ORIGEN Analyzer to rapidly and easily quantitate the levels of murine IgG in a matrix of phosphate-buffered saline, pH 7.4, containing 1% bovine serum albumen (BSA), a typical bioprocess buffer. The assay was formatted using a 1-step, no wash protocol which provides results that are rapid (1 hour incubation time) and precise (<10% C.V.).

MATERIALS AND METHODS

Murine IgG was spiked into a matrix of PBS, pH 7.4, containing 1% BSA. ORIGEN TAG-Goat anti-mouse IgG (IGEN, Inc.) was diluted to 1 µg/ml in PBS, pH 7.8, containing 0.5% normal goat serum, 1.5% Tween 20, and 0.1% SDS. Sheep anti-mouse IgG-coated paramagnetic beads (Dynal, Inc.) were diluted to 0.8 mg/ml in the same diluent. Replicates were prepared with 25 µl of each standard, ruthenylated antibody, and diluted paramagnetic beads and shaken for 1 hour at room temperature. Two hundred-fifty µl of ORIGEN Assay Buffer (IGEN, Inc.) was added to each replicate and the electrochemiluminescence (ECL) was quantitated on the ORIGEN 1.5 Analyzer.

FORMAT



SheepAnti-Mouse IgG Beads

PROTOCOL

- 25 µL Sample
- 25 µL Ruthenylated Antibody
- 25 µL Sheep anti-mouse IgG-coated beads

⇓ Incubate 60 minutes at room temp

- Dilute with 250 µL of Assay Buffer

≠ Read assay

DIRECT ELECTROCHEMILUMINESCENT QUANTITATIVE PCR

MURINE